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An investigation into the solution structures of two self-complementary DNA oligomers, 5′-d(C-G-T-A-C-G) and 5′-d(A-C-G-C-G-C-G-T), by means of nuclear-Overhauser-enhancement measurements

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A 500MHz ^{1}H-n.m.r. study on two self-complementary alternating pyrimidine-purine oligodeoxyribonucleotides, 5′-d(C-G-T-A-C-G) and 5′-d(A-C-G-C-G-C-G-T), is presented. By using the proton–proton nuclear Overhauser effect virtually complete assignments are obtained and a large number of interproton distances [113 in the case of 5′-d(C-G-T-A-C-G) and 79 in the case of 5′-d(A-C-G-C-G-C-G-T)], both intra- and inter-nucleotide, are determined. The interproton-distance data are consistent with an overall right-handed B-DNA-type structure for both oligonucleotides, in agreement with their B-type c.d. spectra. However, whereas 5′-d(C-G-T-A-C-G) adopts a conventional B-type structure with a mononucleotide repeating unit, the interproton-distance data provide evidence that 5′-d(A-C-G-C-G-C-G-T) has a dinucleotide repeating unit consisting of alternation in glycosidic bond and sugar pucker conformations.

With the development of methods for large-scale DNA synthesis (Gassen & Lang, 1982), it has been possible to obtain single-crystal structures of a number of short oligonucleotides comprising examples of the A, B and Z families of DNA (for a review see Dickerson *et al.*, 1982). These have revealed the structural details at atomic resolution of individual base-pairs along the helix heretofore unavailable from fibre diffraction studies, which only yield the averaged structure of a DNA polymer (Arnott & Chandrasekaran, 1981). Such short oligonucleotides are ideally suitable for high-resolution ^{1}H-n.m.r. studies and, consequently, their solution structures can potentially be solved, thereby enabling a comparison of the structures in the crystalline and solution states to be made.

Potentially the most powerful and direct method of conformational analysis in solution is the use of the proton–proton nuclear Overhauser effect (n.O.e.), which can be used to demonstrate the proximity in space of two protons and to determine their separation (Noggle & Schirmer, 1971). This approach has met with considerable success in the study of small proteins (Redfield & Gupta, 1971; Poulsen *et al.*, 1980; Wagner & Wüthrich, 1982), ligand–protein interactions (Gronenborn & Clore, 1982; Clore *et al.*, 1982; Gronenborn *et al.*, 1984*a*), nucleic acid–nucleic acid interactions (Clore *et al.*, 1984) and transfer ribonucleic acids (Roy & Redfield, 1983; Hare & Reid, 1982; Heerschap *et al.*, 1983), and has been applied in a qualitative manner to oligonucleotides, oligonucleotide–drug complexes and DNA polymers (Feigon *et al.*, 1982; Patel, 1982; Patel *et al.*, 1982, 1983; Reid *et al.*, 1983; Scheek *et al.*, 1983; Hare *et al.*, 1983; Gronenborn *et al.*, 1984*b*; Clore & Gronenborn, 1984*a*; Weiss *et al.*, 1984).

As part of a long-term investigation into the solution structures of oligonucleotides and oligonucleotide–protein complexes, we have carried out a 500MHz ^{1}H-n.m.r. study on two short self-complementary DNA oligomers, 5′-d(C-G-T-A-C-G) and 5′-d(A-C-G-C-G-C-G-T). The overall solution structures of both alternating pyrimidine–purine oligonucleotides appears to be that of B DNA, as judged by their c.d. spectra (Kuzmich *et al.*, 1982; G. M. Clore & A. M. Gronenborn, unpublished work). By means of n.O.e. measurements virtually complete assignments are obtained for both oligonucleotides, and a large number of interproton distances, both intra- and inter-nucleotide, are determined. From the distance data, the structures of these two oligonucleotides are solved by model-

Abbreviation used: n.O.e., nuclear Overhauser effect or enhancement.

building. It is shown that, whereas the distance data for the hexamer are compatible with a conventional B DNA structure with a mononucleotide repeat, the distance data for the octamer are suggestive of an alternating B DNA form with a dinucleotide repeating unit. A preliminary account of part of this work has been presented (Clore & Gronenborn, 1983).

Experimental

5'-d(C-G-T-A-C-G) and 5'-d(A-C-G-C-G-C-G-T) were prepared from suitably protected nucleosides by using the phosphite triester method (Matteuci & Caruthers, 1981) as described by Seliger *et al.* (1982) and purified by reverse-phase high-pressure liquid chromatography with a Waters μBondapak C_{18} column.

All n.m.r. spectra were recorded on a Bruker AM500 spectrometer. Spectra in H_2O were recorded by the use of a time-shared hard 1–1 observation pulse (Clore *et al.*, 1983). The n.O.e. values were observed by interleaved difference spectroscopy with a presaturation pulse of 0.3 s, and delays of 2 s between scans to permit relaxation of the system. The power of the selective irradiation pulse used was sufficient to achieve effective instantaneous saturation as regards n.O.e. effects (i.e. the high-power limit) while at the same time maintaining selectivity (Dobson *et al.*, 1982). The estimated relative error in the n.O.e. values, $\Delta N_{ij}/N_{ij}$, is not more than ± 0.15. Assuming an error of ± 0.005 nm in the structure-invariant reference interproton distances, the error in the interproton distance measured from the n.O.e. data is not more than ± 0.02 nm.

Chemical shifts are reported relative to 4,4-dimethylsilapentane-1-sulphonate.

Model-building was carried out with Nicholson skeletal models at a scale of 0.1 nm (1 Å) to 1 cm.

Results and discussion

Resonance assignment

The 500 MHz ^{1}H-n.m.r. spectra of 5'-d(C-G-T-A-C-G) and 5'-d(A-C-G-C-G-C-G-T) in 99.96% ^2H_2O showing only the non-exchangeable proton resonances are shown in Figs. 1(*a*) and 2(*a*). The 500 MHz ^{1}H-n.m.r. spectrum of 5'-d(C-G-T-A-C-G) in 90% H_2O showing both exchangeable and non-exchangeable proton resonances is shown in Fig. 3(*a*). Under the conditions of ionic strength and temperature employed [5°C and 1 M-KCl for 5'-d(C-G-T-A-C-G), and 10°C and 500 mM-KCl for 5'-d(A-C-G-C-G-C-G-T)], both oligonucleotides exist completely in the double-stranded state, as judged from thermal-denaturation studies monitoring both the change in absorbance at 260 nm (Kuzmich *et al.*, 1982) and the change in chemical shifts of the non-exchangeable base protons (results not shown).

The full potential of n.m.r. spectroscopy for structural studies can only be realized after identification of the individual resonance lines. We have made use almost exclusively of the proton–proton n.O.e., which arises from cross-relaxation between protons close in space. For short irradiation times, the magnitude of the n.O.e., N_{ij}, observed on resonance i after irradiation of resonance j is given by:

$$N_{ij} \sim \sigma_{ij} t \qquad (1)$$

as the initial build-up rate of the n.O.e. is equal to the cross-relaxation rate σ_{ij} between the two protons i and j (Wagner & Wüthrich, 1979; Dobson *et al.*, 1982). It will be noted that under these conditions the magnitude of the pre-steady-state n.O.e. observed on proton i after irradiation of proton j is dependent no longer on the total spin-lattice relaxation rate ($1/T_1$) of proton i but only on the cross-relaxation rate between protons i and j. Distance information can then be obtained, as σ_{ij} is inversely proportional to the sixth power of the distance, r_{ij}^6, between the two protons (Solomon, 1955). Thus the ratio of two interproton distances may be obtained from the equation:

$$r_{ij}/r_{kl} = (\sigma_{kl}/\sigma_{ij})^{\frac{1}{6}} = (N_{kl}/N_{ij})^{\frac{1}{6}} \qquad (2)$$

Because $\sigma_{ij} \propto 1/r_{ij}^6$, direct (first-order) pre-steady-state proton–proton n.O.e.s are only detectable up to distances of around 0.5 nm, beyond which effects fall to less than -1% and become virtually undetectable. Moreover, errors introduced by the measurement of the n.O.e. at only a single short irradiation time result in only small errors in the interproton distance ratios. Thus a relative error of say ± 0.2 in the estimates of two n.O.e.s. with values of -30% and -5% results in only an error of ± 0.09 in the value of the calculated distance ratio.

Bearing in mind the relationship between σ_{ij} and r_{ij}, the sequential assignment strategy can now be defined, as only a particular set of n.O.e. values are to be expected for right-handed DNA, as illustrated in Fig. 4, on the basis of the interproton distances derived for classical B and A DNA. Particular attention is drawn to those distance relationships with directional content.

The selective irradiation pulse used in quantitative n.O.e. measurements was applied for 0.3 s, and control experiments with different irradiation times for a few selected resonances indicated that eqn. (1) was valid at this irradiation time (Clore & Gronenborn, 1984*b*). Systematic measurements were carried out by irradiating in turn all non-exchangeable and exchangeable proton resonances

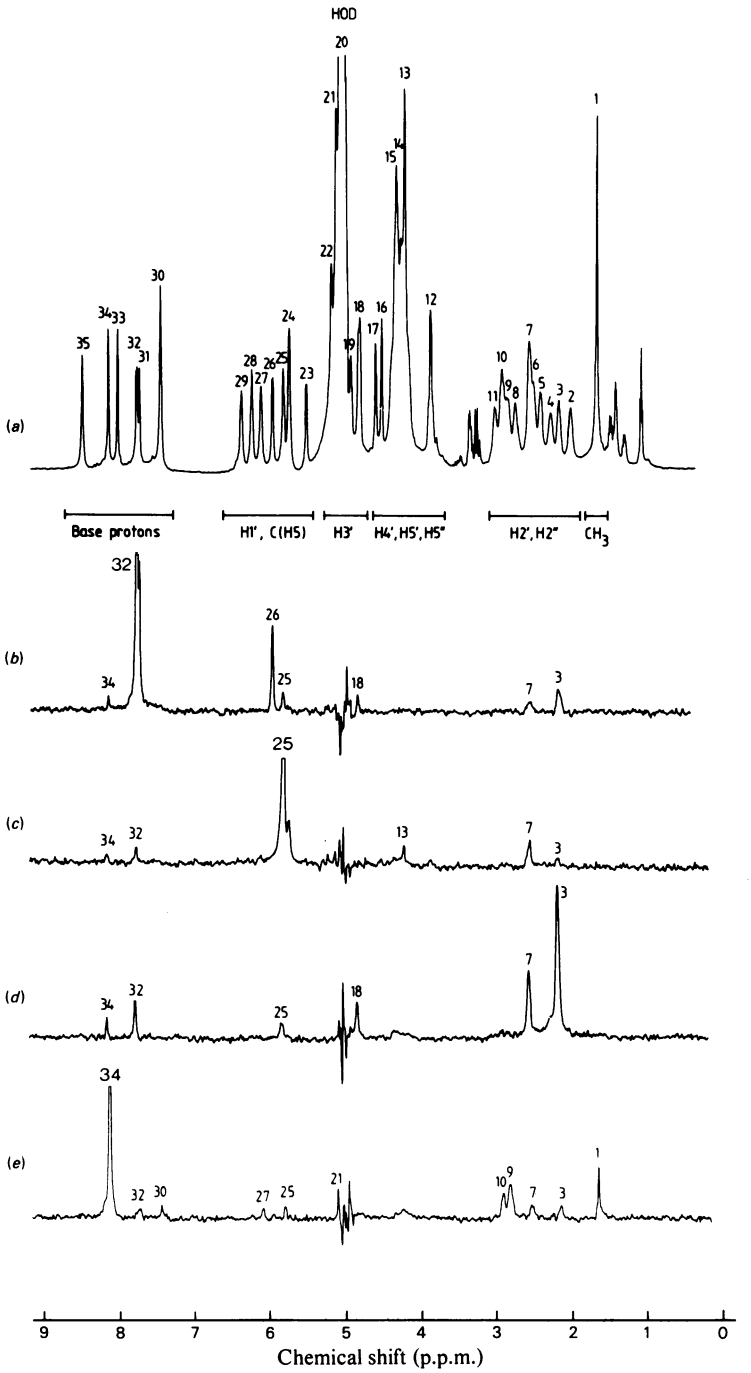

Fig. 1. *Pre-steady-state n.O.e. measurements on double-stranded 5'd-(C-G-T-A-C-G) in 99.96% ^2H_2O at 5°C*

(*a*) The 500 MHz ^{1}H-n.m.r. spectrum between 0 and 9 p.p.m. with all non-exchangeable proton resonances labelled 1–35 (unlabelled resonances are due to residual triethylammonium acetate and other impurities). (*b*)–(*e*) difference spectra (off-resonance minus on-resonance pre-irradiation) after pre-saturation for 0.3 s of: (*b*) the $C_{(1)}$(H6) proton resonance (peak 32) at 7.73 p.p.m.; (*c*) the $C_{(1)}$(H1') proton resonance (peak 25) at 5.79 p.p.m.; (*d*) the $C_{(1)}$(H2') proton resonance (peak 3) at 2.14 p.p.m.; (*e*) the $G_{(2)}$(H8) proton resonance (peak 34) at 8.11 p.p.m. The assignments of the proton resonances are given in Table 1. The experimental conditions are: 0.35 mM double-stranded 5'd(C-G-T-A-C-G) in 99.96% ^2H_2O containing 1 M-KCl, 50 mM-potassium phosphate buffer, pH*6.5 (meter reading uncorrected for the isotope effect on the glass electrode), and 0.1 mM-EDTA. Totals of 1600 and 3200 transients were recorded for the reference and difference n.O.e. spectra respectively.

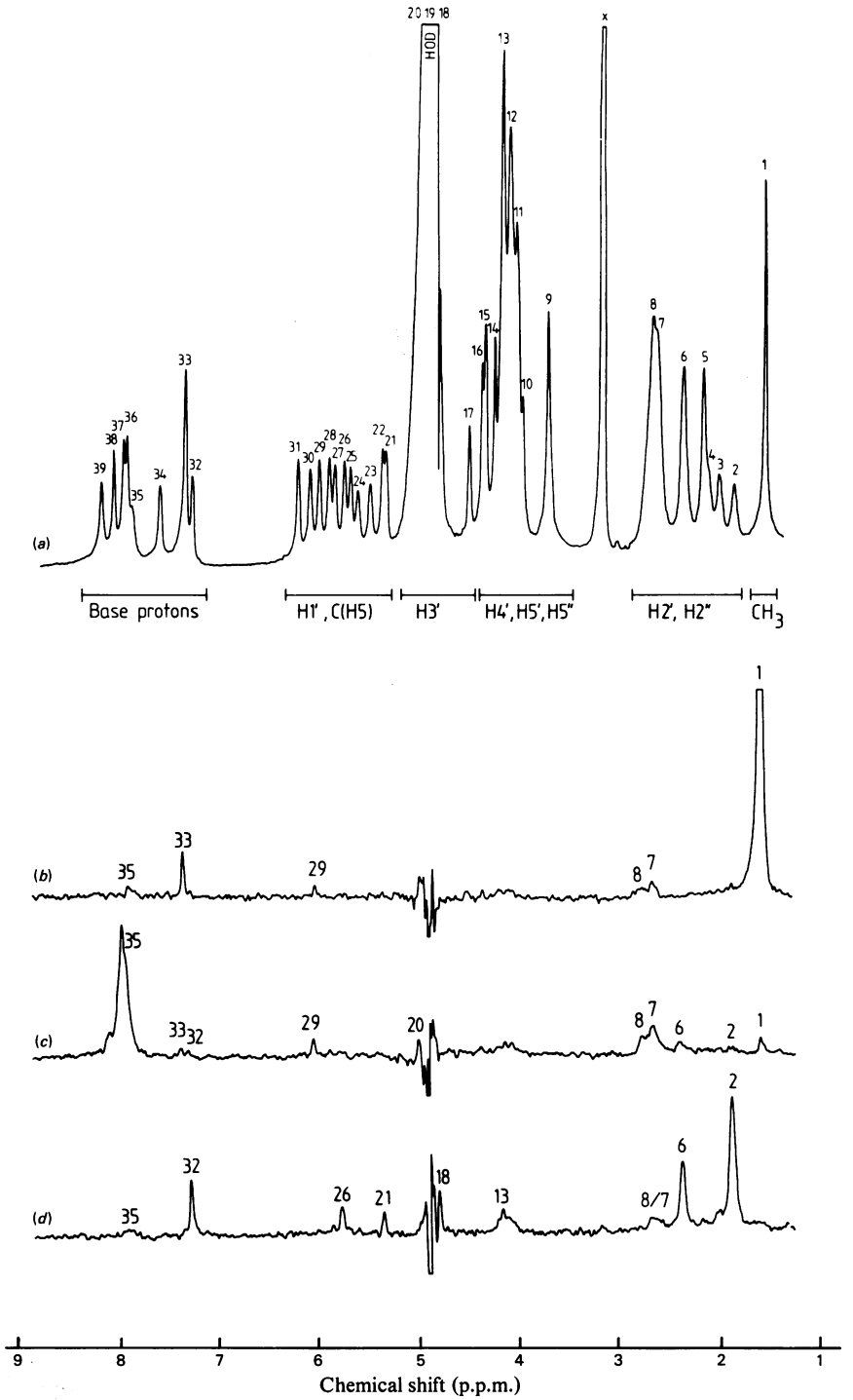

Fig. 2. *Pre-steady-state n.O.e. measurements on double-stranded 5'-d(A-C-G-C-G-C-G-T) in 99.96% ^2H_2O at 10°C*
(*a*) The 500MHz ^{1}H-n.m.r. spectrum between 1 and 9p.p.m. with oligonucleotide resonances labelled 1–39. (The peak marked x is residual triethylammonium acetate). (*b*)–(*d*) Difference spectra (off-resonance minus on-resonance pre-irradiation) after pre-saturation for 0.3s of: (*b*) the $T_{(8)}(CH_3)$ proton resonance (peak 1) at 1.56p.p.m.; (*c*) the $G_{(7)}$(H8) proton resonance (peak 35) at 7.85p.p.m.; (*d*) the $C_{(6)}$(H2') proton resonance (peak 2) at 1.86p.p.m. The assignments of the proton resonances are given in Table 1. The experimental conditions are: 1mM-oligonucleotide in 99.96% ^2H_2O containing 500mM-KCl, 50mM-potassium phosphate buffer, pH*6.5, and 0.01mM-EDTA.

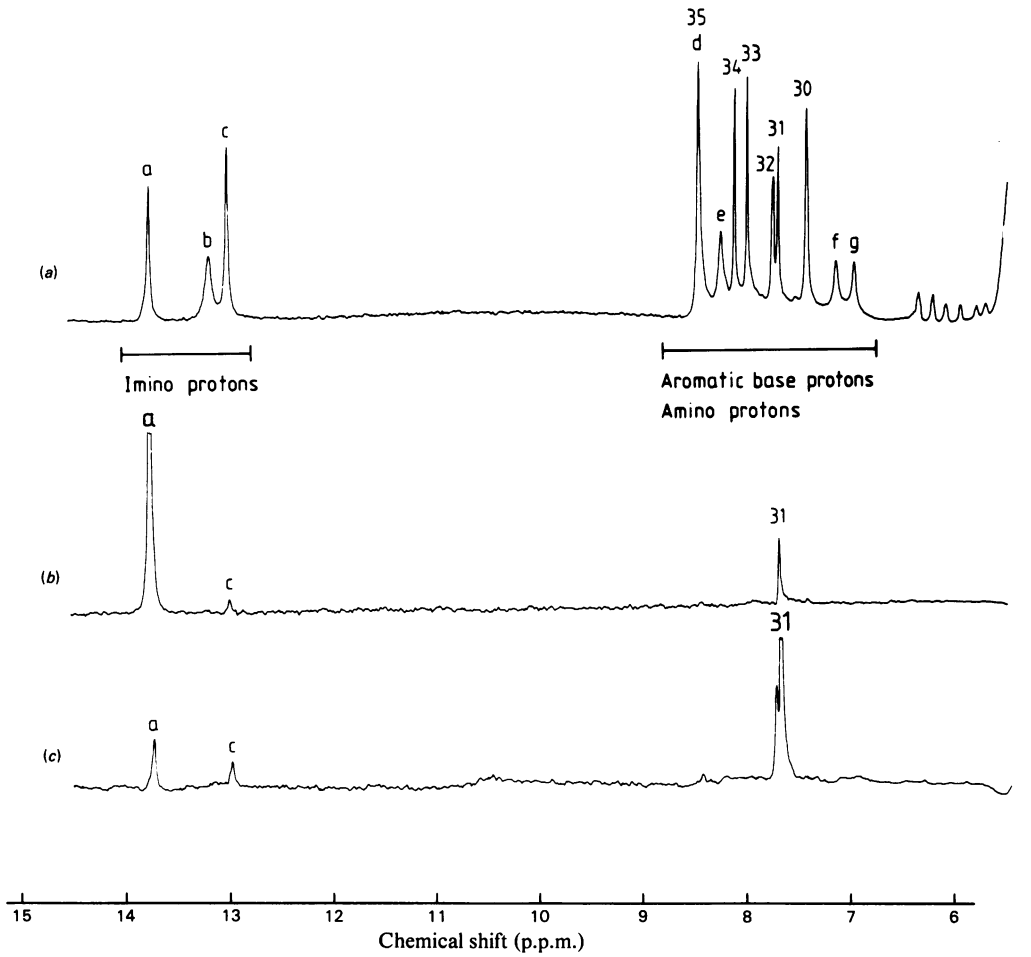

Fig. 3. *Pre-steady-state n.O.e. measurements on double-stranded 5'-d(C-G-T-A-C-G) in 90% H_2O at 5°C*
(*a*) The 500MHz ^{1}H-n.m.r. spectrum between 6 and 15p.p.m. with the exchangeable imino proton resonances labelled a–c, the exchangeable amino proton resonances of bases $C_{(1)}$ and $C_{(5)}$ labelled d–g and the non-exchangeable base proton resonances labelled as in Fig. 1. (It should be noted that the amino proton resonances of the A and G bases are not visible, probably because their exchange rate with water protons is much faster than those of the C bases.) (*b*) and (*c*) Difference spectra (off-resonance minus on-resonance pre-irradiation) after pre-saturation for 0.3s of: (*b*) the $T_{(3)}$(H3) imino proton resonance (peak a) at 13.75p.p.m.; (*c*) the $A_{(4)}$(H2) proton resonance (peak 31) at 7.70p.p.m. The assignments of the proton resonances are given in Table 1. The experimental conditions are as in Fig. 1 except that the sample is in 90% H_2O/10% ^2H_2O. Totals of 800 and 8000 transients were recorded for the reference and difference n.O.e. spectra respectively.

in the case of 5'-d(C-G-T-A-C-G) (labelled 1–35 in Fig. 1*a* and a–g in Fig. 3*a*) and all non-exchangeable proton resonances in the case of 5'-d(A-C-G-C-G-C-G-T) (labelled 1–39 in Fig. 2*a*). N.O.e. measurements on the exchangeable proton resonances of 5'-d(A-C-G-C-G-C-G-T) were not carried out, as the imino proton resonances of the G·C base-pairs were all superimposed in a single peak and the imino proton of the terminal A·T base-pair was not detectable owing to kinetic fraying.

By using the n.O.e. approach, all proton resonances have been assigned unambiguously for both oligonucleotides with the exception of the H5' protons of $T_{(3)}$, $C_{(5)}$ and $G_{(6)}$ in the case of 5'-d(C-G-T-A-C-G) and the H5' and H5" protons of $C_{(2)}$, $G_{(3)}$, $C_{(4)}$, $G_{(5)}$, $C_{(6)}$ and $G_{(7)}$ in the case of 5'-d(A-C-G-C-G-C-G-T). The two sets of assignments are given in Table 1, and the complete set of observed direct first-order n.O.e. values in Tables 2 [5'-d(C-G-T-A-C-G)] and 3 [5'-d(A-C-G-C-G-C-G-T)]. Some examples of pre-steady-state n.O.e. difference spectra are shown in Figs. 1 [5'-d(C-G-T-A-C-G) in ^2H_2O], 2 [5'-d(A-C-G-C-G-C-G-T) in ^2H_2O] and 3 [5'-d(C-G-T-A-C-G) in 90% H_2O]. It goes

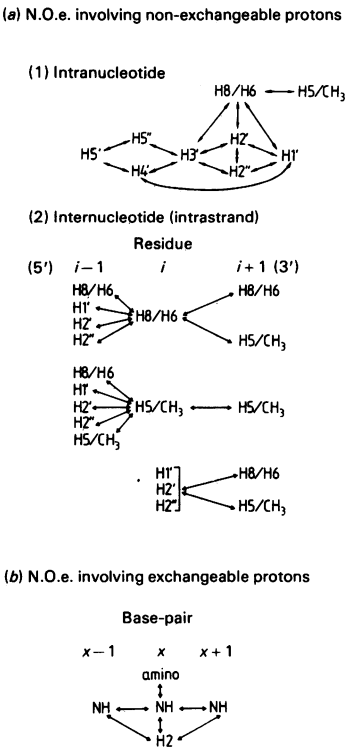

Fig. 4. *Schematic representation of the intra- and internucleotide interproton distances with values not more than 0.5 nm in right-handed DNA, which form the basis of the sequential resonance assignment procedure by means of n.O.e. measurements*
This scheme is applicable to both B and A DNA.

Table 1. *Resonance assignments for double-stranded 5'-d(C-G-T-A-C-G) and 5'-d(A-C-G-C-G-C-G-T)*
Chemical shifts are at 5°C for 5'-d(C-G-T-A-C-G) and 10°C for 5'-d(A-C-G-C-G-C-G-T).

	Chemical shift [p.p.m. (peak no.)]												
	H8/H6	H5/CH_3	H2	H1'	H2'	H2''	H3'	H4'	H5'	H5''	NH	NH_2[1]	NH_2[2]
5'-d(C-G-T-A-C-G)													
$C_{(1)}$	7.66 (32)	5.86 (26)		5.72 (25)	2.07 (3)	2.45 (7)	4.72 (18)	4.10 (13)	3.76 (12)	3.76 (12)		8.20 (e)	7.08 (f)
$G_{(2)}$	8.04 (34)			6.01 (27)	2.74 (9)	2.81 (10)	5.01 (21)	4.41 (16)	4.21 (15)	4.15 (14)	12.93 (c)		
$T_{(3)}$	7.35 (30)	1.56 (1)		5.64 (24)	2.17 (4)	2.45 (7)	4.92 (20)	4.15 (14)		4.10 (13)	13.68 (a)		
$A_{(4)}$	8.38 (35)		7.62 (31)	6.27 (29)	2.81 (10)	2.91 (11)	5.08 (22)	4.49 (17)	4.10 (13)	4.21 (15)			
$C_{(5)}$	7.35 (30)	5.41 (23)		5.64 (24)	1.91 (2)	2.31 (5)	4.82 (19)	4.21 (15)		4.10 (13)		8.40 (d)	6.91 (g)
$G_{(6)}$	7.91 (33)			6.14 (28)	2.63 (8)	2.39 (6)	4.72 (18)	4.21 (15)		4.10 (13)	13.10 (b)		
5'-d(A-C-G-C-G-C-G-T)													
$A_{(1)}$	8.16 (39)		8.04 (38)	6.18 (31)	2.67 (8)	2.67 (8)	4.85 (19)	4.24 (14)	3.71 (9)	3.71 (9)			
$C_{(2)}$	7.57 (34)	5.61 (24)		5.49 (23)	2.12 (4)	2.37 (6)	4.85 (19)	4.16 (13)					
$G_{(3)}$	7.94 (37)			5.89 (28)	2.64 (7)	2.67 (8)	4.95 (20)	4.37 (16)					
$C_{(4)}$	7.32 (33)	5.35 (22)		5.68 (25)	2.01 (3)	2.37 (6)	4.85 (19)	4.16 (13)					
$G_{(5)}$	7.90 (36)			5.83 (27)	2.64 (7)	2.67 (8)	4.95 (20)	4.33 (15)					
$C_{(6)}$	7.25 (32)	5.32 (21)		5.74 (26)	1.86 (2)	2.37 (6)	4.78 (18)	4.16 (13)					
$G_{(7)}$	7.58 (35)			5.99 (29)	2.64 (7)	2.67 (8)	4.95 (20)	4.33 (15)					
$T_{(8)}$	7.32 (33)	1.56 (1)		6.08 (30)	2.17 (5)	2.17 (5)	4.49 (17)	3.96 (10)					

without saying that the assignments based on the n.O.e. data set were thoroughly checked for self-consistency, as this stringent requirement provides a simple and easy check for possible assignment errors.

Interproton-distance determination

In addition to providing assignments by demonstrating the proximity of two protons in space, pre-steady-state n.O.e. measurements allow one to determine interproton distances by using eqn. (2) provided that a reference distance is known and the correlation time of the unknown interproton-distance vector is the same as that of the reference. In this particular case, there are three intranucleotide reference distances that are completely independent of the structure of the DNA, namely $r_{H2'-H2''}$, $r_{C(H6)-C(H5)}$ and $r_{T(H6)-T(CH_3)}$, which, on the basis of standard bond lengths and angles, have values of 0.18, 0.25 and 0.27 nm respectively. [Note that the last-mentioned distance is an average given by $(\langle r_{ij}^{-6} \rangle)^{-\frac{1}{6}}$ calculated on the assumption of free rotation of the methyl group.] In addition,

the distance between the T(H3) and A(H2) protons in a standard Watson–Crick A·T base-pair is 0.29nm; although this distance will not be affected by propellor twisting, it will be dependent on deviations from idealized hydrogen-bond length and geometry. The difficulty, therefore, lies in making the appropriate choice of reference distance in the calculations of the unknown interproton distances, particularly as it is known from relaxation studies on long pieces of DNA that the apparent correlation time of the sugar ring is shorter than that of the bases (Hogan & Jardetzky, 1980). Fortunately, this choice can be based on stereochemical considerations taking into account the expected ranges of the various interproton distances and the expected motions of the different protons based on relaxation measurements and molecular-dynamics calculations (Bolton & James, 1979; Hogan & Jardetzky, 1980; Kollman *et al.*, 1982; Levitt, 1983; Tidor *et al.*, 1983). On this basis we have assumed that: (i) the correlation times for the sugar–sugar, sugar–base (with the exception of the H1′ sugar–base) and sugar–methyl interproton vectors are the same as those of the intranucleotide H2′–H2″ vector; (ii) the correlation times for the base–base and H1′ sugar–base proton vectors are the same as those of the intranucleotide H5–H6 vector; (iii) the correlation times for the base–methyl proton vectors are the same as those of the intranucleotide H6–CH_3 vector. These assumptions seem to be perfectly reasonable, as one would expect that the contribution from internal motion to the correlation times to be dominated by motions within the sugar ring in case (i), by motion about the glycosidic bond in case (ii), and by rotation of the methyl group and motion about the glycosidic bond in case (iii). A check on the validity of assumption (i) can be made by calculating the intranucleotide H1′–H2″ and H2′–H3′ distances with the use of the intranucleotide H2′–H2″ n.O.e. and distance as a reference. Both the H1′–H2″ and H2′–H3′ distances have values of 0.23 ± 0.02nm for all sugar puckers, and the distances calculated from the n.O.e. data all fall within this range (see Tables 2*a* and 3*a*). As expected, the apparent correlation time of the H2′–H2″ vector is significantly shorter than that of the H5–H6 vector, as evidenced by the finding that the H5–H6 intranucleotide distance calculated with the use of the H2′–H2″ n.O.e. and distance as a reference is 0.21nm compared with the idealized value of 0.25nm. This is in complete agreement with the crystallographic data on the native and brominated forms of the B-DNA dodecamer 5′-d(C-G-C-G-A-A-T-T-C-G-C-G), where the temperature factors of the ribose sugars are on average 1.7-fold larger than those of the bases (Drew *et al.*, 1981, 1982). A check on the validity of assumption (ii) can be made by calculating the intra-base-pair T_3(H3)–A_4(H2) distance in 5′-d(C-G-T-A-C-G) with the use of the H5–H6 n.O.e. and distance as a reference: this leads to a value of 0.28nm compared with the idealized value of 0.29nm. In the present case the correlation times for the interproton vectors listed under assumptions (ii) and (iii) are the same within experimental error, indicating that the contribution from free rotation of methyl group to the correlation times of the interproton distance vectors listed under (iii) is negligible. This is easily seen by calculating the intranucleotide H6–CH_3 distance with the use of the H5–H6 n.O.e. and distance as a reference: this leads to calculated distances of 0.27 and 0.26nm for 5′-d(C-G-T-A-C-G) and 5′-d(A-C-G-C-G-C-G-T) respectively, compared with an idealized distance of 0.27nm. We also note that, on the basis of the measured n.O.e. values, the correlation times of the reference interproton distance vectors can safely be assumed to be the same within experimental error for all bases, as the variation in the magnitudes of the intranucleotide H2′–H2″ and H5–H6 n.O.e. values is within a range of not more than $\pm 3\%$ (see also Clore & Gronenborn, 1984*b*).

The intra- and inter-nucleotide distances calculated from the n.O.e. data are given in Tables 2 [5′-d(C-G-T-A-C-G)] and 3 [5′-d(A-C-G-C-G-C-G-T)]. On the basis of an estimated relative error of not more than ± 0.15 in the n.O.e. values and an error of ± 0.005nm in the values of the reference distances, the estimated error in the distance values calculated from the n.O.e. data is not more than ± 0.02nm.

Solution conformations of 5′-d(C-G-T-A-C-G) and 5′-d(A-C-G-C-G-C-G-T)

Given that only a limited number of degrees of freedom are available to double-stranded DNA, the interproton distances determined for 5′-d(C-G-T-A-C-G) (113) and 5′-d(A-C-G-C-G-C-G-T) (79) are sufficient to determine their three-dimensional solution structure with a high degree of confidence. In principle, these structures can be solved by manual model-building. Indeed, by using this approach reasonably accurate values for the glycosidic (χ) and C4′–C3′ (δ) bond torsion angles can be obtained. However, because of potential cumulative errors inherent in such an approach, only qualitative information can be deduced for the other structural parameters, namely the other backbone torsion angles, and the helix twist, helical rise and base tilt. This problem can potentially be overcome by using a non-linear least-squares optimization procedure in which all covalent bond lengths, fixed torsion angles, van der Waal's contacts and hydrogen-bond lengths and geometry are constrained within narrow limits, in order to

Table 2. *Direct pre-steady-state n.O.e. values (irradiation time 0.3s) and interproton distances for double-stranded 5'-d(C-G-T-A-C-G)*

(*a*) Intranucleotide n.O.e. values involving non-exchangeable protons

Proton pair	$C_{(1)}$ N.O.e. (%)	$C_{(1)}$ r_{ij} (nm)	$G_{(2)}$ N.O.e. (%)	$G_{(2)}$ r_{ij} (nm)	$T_{(3)}$ N.O.e. (%)	$T_{(3)}$ r_{ij} (nm)	$A_{(4)}$ N.O.e. (%)	$A_{(4)}$ r_{ij} (nm)	$C_{(5)}$ N.O.e. (%)	$C_{(5)}$ r_{ij} (nm)	$G_{(6)}$ N.O.e. (%)	$G_{(6)}$ r_{ij} (nm)
Intra-sugar n.O.e. values												
[a]H1'–H2'	−5	0.27	−9	0.24	−6	0.26	−8	0.25	−7	0.25	−9	0.24
[a]H1'–H2''	−15	0.22	−18	0.22	−14	0.23	−15	0.22	−16	0.22	−20	0.21
[a]H1'–H4'	−3[f]	0.29	−4[f]	0.28[f]	−3[f]	0.29[f]	−3[f]	0.29[f]	−3[f]	0.29[f]	−3[f]	0.29[f]
[a]H2'–H2''	−59	0.18	−59	0.18	−60	0.18	−59	0.18	−57	0.18	−58	0.18
[a]H2'–H3'	−15	0.22	−14	0.23	−17	0.22	−17	0.22	−19	0.21	−20	0.21
[a]H2''–H3'	−11	0.24	−12	0.23	−11	0.24	−8	0.25	−13	0.23	−15	0.22
[a]H2''–H4'	−2[f]	0.31[f]	−3[f]	0.29[f]	−3[f]	0.29[f]	−2[f]	0.31[f]	−3[f]	0.29[f]	−2[f]	0.31[f]
[a]H3'–H4'	−5[e]	0.27[e]	−8	0.25	−8	0.25	−6	0.26	−7	0.25	−5	0.27
[a]H3'–H5''	−10	0.24	−9	0.24	−20	0.21	−6	0.26	−10	0.24	−9[e]	0.24[e]
[a]H4'–H5''	−8[d]	0.25	−18	0.22			−19	0.22				
[a]H4'–H5'	−8[d]	0.25	−12	0.23			−12	0.23				
Sugar–base n.O.e. values												
[b]H1'–H6/H8	−2	0.38	−2	0.38	−2	0.38			−3	0.38	−2	0.38
[a]H2'–H6/H8	−13	0.23	−20	0.21	−19	0.22	−19	0.22	−16	0.22	−19	0.21
[a]H3'–H6/H8	−2	0.31	−3	0.29	−5	0.27	−2	0.31	−4	0.28	−1	0.35
Intra-base n.O.e. values												
[b]H6–H5	−26	0.25							−23	0.25		
[c]H6–CH_3					−13	0.27						

(*b*) Internucleotide n.O.e. values involving non-exchangeable protons

Proton of 5'-nucleotide	Proton of 3'-nucleotide	$C_{(1)}pG_{(2)}$ N.O.e. (%)	$C_{(1)}pG_{(2)}$ r_{ij} (nm)	$G_{(2)}pT_{(3)}$ N.O.e. (%)	$G_{(2)}pT_{(3)}$ r_{ij} (nm)	$T_{(3)}pA_{(4)}$ N.O.e. (%)	$T_{(3)}pA_{(4)}$ r_{ij} (nm)	$A_{(4)}pC_{(5)}$ N.O.e. (%)	$A_{(4)}pC_{(5)}$ r_{ij} (nm)	$C_{(5)}pG_{(6)}$ N.O.e. (%)	$C_{(5)}pG_{(6)}$ r_{ij} (nm)
[b]H6/H8	H8/H6	−2	0.36	−3	0.34	−2[d]	0.37	−2	0.37	−2	0.39
[b]H8	H5							−3	0.35		
[c]H8	CH_3			−8	0.30						
[c]CH_3	H8					$\leqslant -0.5$	$\geqslant 0.47$				
[a]H1'	H8/H6	−1	0.33	−3	0.29	−2	0.32	−3	0.29	−2	0.32
[a]H2'	H8/H6	−5	0.27	−10	0.24	−4	0.28	−8[d]	0.25	−5	0.27
[a]H2''	H8/H6	−7	0.26	−8[d]	0.25	−6	0.26	−10	0.24	−7	0.25
[a]H2'	CH_3			−10	0.24						
[a]H2''	CH_3			−9	0.24						

Table 2 (continued)

(*c*) Intra- and inter-base-pair n.O.e. values involving exchangeable protons

	Intra-base-pair						Inter-base-pair			
Proton pair	N.O.e (%)	r_{ij} (nm)	N.O.e (%)	r_{ij} (nm)	N.O.e (%)	r_{ij} (nm)	N.O.e (%)	r_{ij} (nm)	N.O.e (%)	r_{ij} (nm)
[b]G(H1)–G(H1)/T(H3)							−3	0.35	−3	0.35
[b]G(H1)–A(H2)									−5[g]	0.32
[b]T(H3)–A(H2)					−12[g]	0.28				
[b]G(H1)–CH_2^1)[h]	−15	0.27	−14	0.27						
[g]C(NH_2^1)–C(NH_2^2)[h]	−100	0.18[i]	−100	0.18[i]						

[a]These interproton distances were calculated with the use of the H2′–H2″ n.O.e. and distance (0.178 nm) as a reference. The mean value of the H2′–H2″ n.O.e. for the six sugars is $-59\pm1\%$.

[b]These interproton distances were calculated with the use of the C(H6)–C(H5) n.O.e. and distance (0.246 nm) as a reference. The mean value of the C(H6)–C(H5) n.O.e. for the $C_{(1)}$ and $C_{(5)}$ bases is $-24.5\pm2\%$.

[c]These interproton distances were calculated with the use of the $T_{(3)}$(H6)–$T_{(3)}$(CH_3) n.O.e. (−13%) and distance (0.27 nm) as a reference.

[d]The following pairs of proton resonances are superimposed: $T_{(3)}$(H6) and $C_{(5)}$(H6) in peak 30, $T_{(3)}$(H1′) and $C_{(5)}$(H1′) in peak 24, $G_{(2)}$(H2″) and $A_{(4)}$(H2′) in peak 10, and $C_{(1)}$(H5″) and $C_{(1)}$(H5′) in peak 12. Consequently, the individual values for the following pairs of n.O.e. measurements cannot be resolved, as only a single n.O.e. is observed for each pair: the $T_{(3)}$(H6)–$T_{(3)}$(H1′) and $C_{(5)}$(H6)–$C_{(5)}$(H1′) n.O.e. pair, the $T_{(3)}$(H6)–$A_{(4)}$(H8) and $A_{(4)}$(H8)–$C_{(5)}$(H6) n.O.e. pair, the $G_{(2)}$(H2″)–$T_{(3)}$(H6) and $A_{(4)}$(H2′)–$C_{(5)}$(H6) n.O.e. pair, and the $C_{(1)}$(H4′)–$C_{(1)}$(H5″) and $C_{(1)}$(H4′)–$C_{(1)}$(H5′) n.O.e. pair. In the Table, the contributions from the two members of each pair to the observed n.O.e. are assumed to be equal.

[e]It will be noted that the resonances $C_{(1)}$(H3′) and $G_{(6)}$(H3′) are superimposed in peak 18 and resonances $C_{(1)}$(H4′) and $G_{(6)}$(H5″) in peak 13. As a result, the n.O.e. of −14% observed on peak 18 after irradiation of peak 13 (and vice versa) cannot be partitioned *a priori* between the $C_{(1)}$(H3′)–$C_{(1)}$(H4′) n.O.e. and the $G_{(6)}$(H3″)–$G_{(6)}$(H5″) n.O.e. However, the distance $r_{C_{(1)}(H3')-C_{(1)}(H4')}$ can be measured directly from a model on the basis of the sugar pucker conformation deduced from other interproton distances (see the text for discussion). From the $r_{C_{(1)}(H3')-C_{(1)}(H4')}$ distance of 0.27 nm obtained in this way, the expected value of the $C_{(1)}$(H3′)–$C_{(1)}$(H4′) n.O.e. can be calculated to be −5%, and consequently the value of the $G_{(6)}$(H3′)–$G_{(6)}$(H5″) n.O.e. can be deduced (−9%) and the distance $r_{G_{(6)}(H3')-G_{(6)}(H5'')}$ estimated (0.24 nm).

[f]The relative error, $\Delta N/N$, on these n.O.e. values is not more than ±0.30. However, as the values of the n.O.e. measurements lie in the 2–4% range, the error on the distance calculated from them is still small (not more than ±0.03 nm).

[g]The values of the $T_{(3)}$(H3)–$A_{(3)}$(H2) and $G_{(2)}$(H1)–$A_{(4)}$(H2) n.O.e. measurements given in the Table are those for the n.O.e. values observed on the $T_{(3)}$(H3) and $G_{(2)}$(H1) imino proton resonances respectively after irradiation of the $A_{(4)}$(H2) proton resonance (see the text for discussion).

[h]The superscripts 1 and 2 refer to the hydrogen-bonded proton and the non-hydrogen-bonded proton respectively of the $-NH_2$ group. The amino protons are assigned to the C base rather than the G base, as the distance calculated from the values of the intra-base-pair n.O.e. between the imino and NH_2^1 amino protons is only consistent with the distance between the G(H1) imino proton and the C(NH_2^1) amino proton (approx. 0.26 nm) and is inconsistent with the distance between the G(H1) imino proton and the G(NH_2^1) amino proton (approx. 0.23 nm).

[i]The interproton distance between the two protons of the $-NH_2$ group was calculated on the basis of standard bond lengths and angles.

Table 3. *Direct pre-steady-state n.O.e. values (irradiation time 0.3s) and interproton distances for double-stranded 5'-d(A-C-G-C-G-C-G-T)*

(*a*) Intranucleotide n.O.e. values

Proton pair	$A_{(1)}$ N.O.e. (%)	$A_{(1)}$ r_{ij} (nm)	$C_{(2)}$ N.O.e. (%)	$C_{(2)}$ r_{ij} (nm)	$G_{(3)}$ N.O.e. (%)	$G_{(3)}$ r_{ij} (nm)	$C_{(4)}$ N.O.e. (%)	$C_{(4)}$ r_{ij} (nm)	$G_{(5)}$ N.O.e. (%)	$G_{(5)}$ r_{ij} (nm)	$C_{(6)}$ N.O.e. (%)	$C_{(6)}$ r_{ij} (nm)	$G_{(7)}$ N.O.e. (%)	$G_{(7)}$ r_{ij} (nm)	$T_{(8)}$ N.O.e. (%)	$T_{(8)}$ r_{ij} (nm)
Intra-sugar n.O.e. values																
[a]H1′–H2′	−30[d]		−10	0.24	−20	0.21	−10	0.23	−18	0.21	−10	0.23	−13	0.23	−26[d]	
[a]H1′–H2″			−20	0.21	−23	0.21	−23	0.21	−20	0.21	−23	0.21	−20	0.21		
H1′–H4′	−3[i]		−8[i]		−6[i]		−8[i]		−7[i]		−7[i]		−6[i]		−7[i]	
[a]H2′–H2″	[d]		−53	0.18	[e]		−56	0.18	[e]		−51	0.18	[e]		[d]	
[a]H2′–H3′	−8[d]		−7		9		−11	0.23	[g]		−15	0.22	[g]		−17[d]	
[a]H2″–H3′			[f]		[g]		[f]		[g]		−16	0.22	[g]			
H2″–H4′	−9		[f]		−7[i]		[f]		[g]		[f]		[g]		−7[i]	
[a]H3′–H4′	−4	0.27	[f]		−8	0.24	[f]		[g]		−12[i]		[g]		−10	0.23
H4′–H5″	−25		[h]				[h]				[h]		[h]		[h]	
H4′–H5′																
H3′–H5″	−5															
Sugar–base n.O.e. values																
[b]H1′–H6/H8	−10	0.29	−5	0.32	−5	0.32	−5	0.32	−6	0.31	−5	0.32	−6	0.31	−5	0.32
[a]H2′–H6–H8	−24	0.20	−21	0.20	−31	0.20	−26	0.20	−30	0.20	−25	0.20	−15	0.22	−20	0.21
[a]H3′–H6/H8	−1	0.35	−2	0.31	−4	0.27	−2	0.31	−6	0.26	−6	0.26	−5	0.26	−7	0.25
Intra-base n.O.e. values																
[b]H6–H5			−22	0.25			−24	0.25			−25	0.25				
[c]H6–CH_3															−18	0.27

Table 3 (continued)

(*b*) Internucleotide n.o.e. values

Proton of 5′-nucleotide	Proton of 3′-nucleotide	$A_{(1)}pC_{(2)}$ N.O.e. (%)	$A_{(1)}pC_{(2)}$ r_{ij} (nm)	$C_{(2)}pG_{(3)}$ N.O.e. (%)	$C_{(2)}pG_{(3)}$ r_{ij} (nm)	$G_{(3)}pC_{(4)}$ N.O.e. (%)	$G_{(3)}pC_{(4)}$ r_{ij} (nm)	$C_{(4)}pG_{(5)}$ N.O.e. (%)	$C_{(4)}pG_{(5)}$ r_{ij} (nm)	$G_{(5)}pC_{(6)}$ N.O.e. (%)	$G_{(5)}pC_{(6)}$ r_{ij} (nm)	$C_{(6)}pG_{(7)}$ N.O.e. (%)	$C_{(6)}pG_{(7)}$ r_{ij} (nm)	$G_{(7)}pT_{(8)}$ N.O.e. (%)	$G_{(7)}pT_{(8)}$ r_{ij} (nm)
[b]H8	H5	—		−2	0.37	−4	0.33	−3	0.35	−3	0.35	−4	0.33	−3	0.35
[c]H8	CH_3	−2	0.39	—		−7	0.32			−5	0.34	—			
[b]H1′	H8/H6	−4	0.33	−3	0.35	−8	0.30	−5	0.32	−7	0.30	−1	0.42	−5	0.31
[a]H2′	H8/H6	−16[d]		−3	0.29			−9	0.24	−13	0.22	−4	0.27		
[a]H2″	H8/H6			−12	0.23			−13	0.22	−18	0.21	−9			
[a]H2′	CH_3													−5	0.26
[a]H2″	CH_3													−3	0.29
[a]H2′	H5	−7[d]				−9	0.24			−9	0.24				
[a]H2″	H5					−8	0.25			−7	0.25				
[a]H2″	H2′			[h]				[h]				[h]			
[a]H2″	H3′			[h]				[h]				[h]			

[a] These interproton distances were calculated with the use of the H2′–H2″ n.O.e. and distance (0.178 nm) as a reference. The mean value of the three measured H2′–H2″ n.O.e. values is $-53 \pm 2\%$.

[b] These interproton distances were calculated with the use of the C(H6)–C(H5) n.O.e. and distance (0.246 nm) as a reference. The mean value of the C(H6)–C(H5) n.O.e. for the three C bases is $-24 \pm 2\%$.

[c] These interproton distances were calculated with the use of the $T_{(8)}(CH_3)$–$T_{(8)}$(H6) n.O.e. (−18%) and distance (0.27 nm) as a reference.

[d] The H2′ and H2″ resonances of $A_{(1)}$ are superimposed in peak 8 and the H2′ and H2″ resonances of $T_{(8)}$ in peak 5. Consequently the n.O.e. between the H2′ and H2″ protons for $A_{(1)}$ and $T_{(8)}$ cannot be measured. In addition, the n.O.e. values observed between peaks 8 and 31 [$A_{(1)}$(H1′)] (−30%), peaks 5 and 30 [$T_{(8)}$(H1′)] (−26%), peaks 8 and 34 [$C_{(2)}$(H6)] (−16%) and peaks 8 and 24 [$C_{(2)}$(H5)] (−7%) cannot be resolved into individual contributions from the H2′ and H2″ protons, although they enable assignments to be made.

[e] The H2′ and H2″ resonances of $G_{(3)}$, $G_{(5)}$ and $G_{(7)}$ are too close together (approx. 15 Hz) to enable one to measure the n.O.e. between the H2′ and H2″ protons for these three nucleotides.

[f] The H2″ resonances of $C_{(2)}$, $C_{(4)}$ and $C_{(6)}$ are superimposed in peak 6, the H3′ resonances of $C_{(2)}$ and $C_{(4)}$ in peak, 19, and the H4′ resonances of $C_{(2)}$, $C_{(4)}$ and $C_{(6)}$ in peak 13. Consequently, the observed n.O.e. values between peaks 6 and 19 (−10%), between peaks 6 and 13 (−10%) and between peaks 19 and 13 (−6%) cannot be resolved into individual contributions, although they enable assignments to be made.

[g] The H2″ resonances of $G_{(3)}$ and $G_{(5)}$ and the H2′ resonance of $G_{(7)}$ are superimposed in peak 8, the H2′ resonances of $G_{(3)}$ and $G_{(5)}$ and the H2″ resonance of $G_{(7)}$ in peak 7, the H3′ resonances of $G_{(3)}$, $G_{(5)}$ and $G_{(7)}$ in peak 20, and the H4′ resonances of $G_{(5)}$ and $G_{(7)}$ in peak 15. Consequently, the observed n.O.e. values between peaks 7 and 20 (−13%), between peaks 8 and 20 (−11%), between peaks 8 and 15 (−7%) and between peaks 20 and 15 (−8%) cannot be resolved into individual contributions, although they enable assignments to be made.

[h] The n.O.e. values observed between peaks 6 (H2″ resonances of $C_{(2)}$, $C_{(4)}$ and $C_{(6)}$) and peaks 7/8 (purine H2′/H2″ resonances) and between peaks 6 and 20 (H3′ resonances of $G_{(3)}$, $G_{(5)}$ and $G_{(7)}$), of −8% and −3% respectively, are only compatible with n.O.e. values between the H2″ protons of $C_{(2)}$, $C_{(4)}$ and $C_{(6)}$ and the H2′ and H3′ protons of the G base on their 3′ side.

[i] These n.O.e. values were difficult to quantify and their relative error, $\Delta N/N$ is approx. ± 0.5, so no attempt was made to calculate distances from them.

refine an initial trial model on the basis of the interproton-distance data. In the present paper, however, we have restricted ourselves to information derived from manual model-building.

The minimum requirement to define both the glycosidic bond torsion angle χ and the sugar pucker conformation expressed in terms of the C4′–C3′ bond torsion angle δ is two distance ratios: namely one distance ratio involving any two of the three intranucleotide sugar–base interproton distances $r_{H1'-H8/H6}$, $r_{H2'-H8/H6}$ and $r_{H3'-H8/H6}$, and one of the two distance ratios $r_{H1'-H2''}/r_{H1'-H2'}$ and $r_{H2'-H3'}/r_{H2''-H3'}$ involving intranucleotide sugar–sugar interproton distances. This information is available for all the nucleotide residues of both oligonucleotides with the exception of residues $A_{(1)}$ and $T_{(8)}$ of 5′-d(A-C-G-C-G-C-G-T). However, even for these two residues χ and δ can be defined within a relatively narrow range ($\pm 20°$), as two intranucleotide sugar–base interproton distance ratios are available.

By following this approach we find that there is no significant difference in the nucleotide conformations of the residues of 5′-d(C-G-T-A-C-G): the glycosidic bond conformations are all anti with χ restricted to the relatively narrow range of $-110 \pm 10°$, and the sugar pucker conformations all lie in the C1′-exo range with the values of $115 \pm 10°$ for δ. This compares favourably with both the single-crystal data on the B-DNA dodecamer 5′-d(C-G-C-G-A-A-T-T-C-G-C-G), where $\chi_{mean} = -117 \pm 14°$ and $\delta_{mean} = 123 \pm 21°$ (Dickerson & Drew, 1981), and the fibre diffraction data on B DNA where $\chi = -102°$ and $\delta = 139°$ (Arnott *et al.*, 1980).

The situation in the case of 5′-d(A-C-G-C-G-C-G-T), however, is somewhat different. We find that, whereas the pyrimidine nucleotides adopt a conventional anti conformation ($\chi = -100 \pm 10°$) with a C1′-exo sugar pucker ($\delta = 125 \pm 10°$), the purine nucleotides appear to adopt a high anti conformation ($\chi = -70 \pm 10°$) with an O1′-endo sugar pucker ($\delta = 105 \pm 10°$). The variation in sugar pucker can be deduced on qualitative grounds alone on the basis of the intranucleotide H1′–H2′ and H1′–H2″ n.O.e. values given in Table 3(*a*): whereas the H1–H2′ n.O.e. values are approximately half the magnitude of the H1′–H2″ n.O.e. values in the case of the pyrimidine nucleotides, indicative of an S-type conformation (i.e. in the C1′-exo/C2′-endo range), they are approximately equal in the case of the purine nucleotides, indicative of an N-type conformation (i.e. in the O1′-endo/C3′-endo range). These findings suggest that 5′-d(A-C-G-C-G-C-G-T) adopts an alternating B-DNA structure with a dinucleotide repeat in solution. An alternating B-DNA model has been proposed by Klug *et al.* (1979), and more recently an alternating B-type structure for poly[d(G-C)], known as wrinkled B DNA, has been observed by fibre diffraction (Arnott *et al.*, 1983). In both these structures the pyrimidine nucleotides show a conventional anti conformation ($\chi \sim -105°$) with a C2′-endo sugar pucker ($\delta \sim 145°$) similar to that found for 5′-d(A-C-G-C-G-C-G-T). In the Klug model, the purine nucleotides have a low anti conformation ($\chi = -147°$) and an O1′-endo sugar pucker ($\delta = 99°$) similar to that of conventional A DNA ($\chi = -154°$ and $\delta = 83°$). In wrinkled B DNA, however, the purine nucleotides have a high anti conformation ($\chi = -86°$) and a C2′-endo sugar pucker ($\delta = 148°$). Thus the purine nucleotides of 5′-d(A-C-G-C-G-C-G-T) adopt a glycosidic-bond conformation similar to that of wrinkled B DNA and a sugar pucker conformation similar to that of the Klug alternating B-DNA model.

The C5′–C4′-bond torsion angle γ can also be uniquely defined provided that two out of the three intranucleotide distances $r_{H3'-H5''}$, $r_{H4'-H5''}$ and $r_{H4'-H5'}$ are known. This information is only available for residues $G_{(2)}$ and $A_{(4)}$ of 5′-d(C-G-T-A-C-G), where simple model-building indicates that γ lies in the g^+ range with a value of $50 \pm 10°$. For the other residues of 5′-d(C-G-T-A-C-G) only the distance $r_{H3'-H5''}$ is available, and for the residues of 5′-d(A-C-G-C-G-C-G-T) none of these three distances could be measured. Nevertheless, it seems likely that γ lies in the g^+ range ($60 \pm 60°$) for these residues, as no n.O.e. could be observed from the H8/H6 base protons to the H5′ or H5″ sugar protons, indicative of a separation of more than 0.4nm; in the t or g^- ranges, the separation between the H8/H6 base proton and the H5″ and H5′ protons respectively would be less than 0.4nm for the glycosidic-bond and sugar pucker conformations determined above. These findings are in complete agreement with the single-crystal and fibre diffraction data on right-handed DNA (Dickerson & Drew, 1981; Shakked *et al.*, 1983; Arnott & Hukins, 1972; Arnott & Chandrasekaran, 1981; Arnott *et al.*, 1983).

Once the glycosidic-bond and sugar pucker conformations are known for each nucleotide, the inter-residue interproton distances enable one to define the position of each base-pair with respect to that of the adjacent base-pairs on either side in terms of approximate values of the helical rise, helical twist and base tilt. The handedness of the helix can be immediately deduced from the internucleotide n.O.e. data on the basis of qualitative considerations alone: namely the observation of n.O.e.s from the C(H5) and $T(CH_3)$ protons to the H8 proton of the purine base on their 5′ side (indicating a separation of not more than 0.4nm between these protons) and the absence of detect-

able n.O.e.s from the C(H5) and T(CH_3) protons to the H8 proton of the purine on their 3′ side (indicating a separation of more than 0.4nm between these protons) is only compatible with a right-handed helix. Manual model-building on the basis of these internucleotide distances results in right-handed helical models with an average helical rise of 0.34 ± 0.05nm, an average helical twist of $36 \pm 10°$ and an average base tilt of $0 \pm 10°$ (i.e. the base-pairs approximately perpendicular to the helix axis). These findings are in complete agreement with the single-crystal and fibre diffraction data on B DNA (Dickerson & Drew, 1981; Arnott & Hukins, 1972). It should also be noted that the large difference in base tilt between B (approx. 4°) and A (approx. 18°) DNA arises primarily as a result of the difference in sugar pucker (O1′-endo to C2′-endo range compared with pure 3′-endo) and glycosidic-bond (anti compared with low anti) conformations.

The inter-residue interproton distances involving the imino protons of 5′-d(C-G-T-A-C-G) deserve some additional comment. The separation of 0.35nm between the imino protons of the first base-pair step $C_{(1)} \cdot G_{(6)}$–$G_{(2)} \cdot C_{(5)}$ compares well with the value of 0.38nm for such a step in classical B DNA but poorly with that of 0.46nm for classical A DNA (derived from the fibre diffraction co-ordinates given by Arnott & Hukins, 1972). The separation of 0.35nm between the imino protons of the second base-pair step $G_{(2)} \cdot C_{(5)}$–$T_{(3)} \cdot A_{(4)}$ is similar to that for the same step in both classical B (0.35nm) and A (0.34nm) DNA. However, the separation of 0.32nm between the $A_{(4)}$(H2) and $G_{(2)}$(H1) protons in the second base-pair step is much smaller than that expected for either classical B (0.38nm) or A (0.45nm) DNA. That the separation between the $A_{(4)}$(H2) and $G_{(2)}$(H1) protons is smaller than that between the $T_{(3)}$(H3) and $G_{(2)}$(H1) imino protons can be immediately deduced by inspection of the n.O.e. shown in Fig. 3: the magnitude of the n.O.e. observed on the $G_{(2)}$(H1) imino proton resonance (peak c) after irradiation of the $A_{(4)}$(H2) resonance (peak 31, Fig. 3*c*) is approximately double that after irradiation of the $T_{(3)}$(H3) imino proton resonance (peak a, Fig. 3*b*). This finding can be interpreted in terms of propellor twisting of the central A·T base-pairs.

Turning to the internucleotide n.O.e. values for 5′-d(A-C-G-C-G-C-G-T) (see Table 3*b*) we note that small n.O.e. values are observed between peaks 6 (H2″ resonances of $C_{(2)}$,$C_{(4)}$ and $C_{(6)}$) and peaks 7/8 (purine H2′/H2″ resonances) and between peaks 6 and 20 (H3′ resonances of $G_{(3)}$, $G_{(5)}$ and $G_{(7)}$). These n.O.e. values are only compatible with n.O.e.s between the H2″ protons of the pyrimidine residues, $C_{(2)}$, $C_{(4)}$ and $C_{(6)}$, and the H2′ and H3′ protons of the G base on their 3′ side. No equivalent n.O.e. values are observed for the GpC steps. Although clearly fragmentary and of no quantitative value, these n.O.e. values provide additional qualitative evidence for a dinucleotide repeat in 5′-d(A-C-G-C-G-C-G-T).

Concluding remarks

In the present paper we have demonstrated the potential of proton–proton n.O.e. measurements to probe the solution structures of small oligonucleotides and define the glycosidic-bond and sugar pucker conformations of the individual residues. The present data are consistent with a conventional right-handed B-DNA-type structure for 5′-d(C-G-T-A-C-G) with a mononucleotide repeating unit. In the case of 5′-d(A-C-G-C-G-C-G-T) the data have provided evidence that a dinucleotide repeating unit consisting of alternation in glycosidic-bond and sugar pucker conformations is superimposed on an overall B-DNA-type structure.

The n.O.e. measurements in solution cannot compete as yet with single-crystal X-ray-diffraction data in the wealth of structural information that they can provide. This is because the n.O.e. data are only capable of providing an interproton-distance data set between protons separated by less than 0.5nm, whereas high-resolution single-crystal X-ray diffraction is able to locate the positions of all atoms in space with the exception of protons. Nevertheless, the data obtained by the two techniques should be treated as complementary. This is particularly so when the different natures of the solution and crystal structures are borne in mind. Both are average structures with small time-dependent fluctuations about a mean, as oligonucleotides exhibit some degree of flexibility. However, whereas the crystal structure is a simple linear superposition of all the populations present in the crystal, the distances obtained from n.O.e. measurements in solution are $(\langle r_{ij}^{-6} \rangle)^{-\frac{1}{6}}$ means. Thus, whereas all fluctuations are equally weighted in the crystal structure, the average distance between two protons in the solution structure is weighted in favour of the fluctuations with the shorter interproton distances. In addition, the molecules in the crystal are subject to crystal packing forces, and these, for example, probably account for the asymmetry in the crystal structure of the self-complementary dodecamer solved by Dickerson & Drew (1981). With these considerations taken into account, the interproton distances obtained from n.O.e. measurements should provide a powerful tool supplementing crystallographic studies, particularly in cases where crystal data are not available, in comparative studies of

oligonucleotides with an array of different sequences, and in the study of transitions between different conformational states of DNA.

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